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User Manual

Version 1.0

ARS-Media

Ion Solution Calculator

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Overview

The ARS-Media program functions as a type of calculator to generate the type and quantity of salts required to achieve target ion levels specified by the user. The term “salts” is used to refer to salts, acids, and bases. The core of the program is a minimizing linear programming (LP) module. The program will run on computers with the Windows operating system (e.g., 2000, XP). Details of why and how the LP equations are constructed are explained by Niedz and Evens (2006).

Niedz, R.P. and Evens, TJ. A solution to the problem of ion confounding in experimental biology. Nature Methods 6:417, 2006.

Any studies that use ARS-Media should cite the software version number and the reference above.

Installation Instructions

To install ARS-Media: 1) Open the folder named "Installer", 2) Double-click on "setup.exe" and follow the prompts. The program will be installed on your computer in the “Program Files” directory.

Quick Start

1. Enter a name for the experiment.

2. Go to the Salt Selection Screen and select all or some of the salts in the database. The OFC value is used to weight the importance of the salt in deriving an LP solution – the lower the number the more important the salt. This is because the LP is minimizing the solution. The initial default OFC value is 1 for all salts; therefore, all selected salts are considered equally if no changes are made. If stock solutions are available the concentration may be entered; otherwise, the default value is 1 mg/ml.

3. Go to the Define Ion Target Screen and enter an amount for each ion. The default is 0 mM. The “Make Media” button on the main panel remains grayed out until at least one ion value is entered.

4. Return to the main panel and select “Make Media.” A solution is generated for a default volume of 1 liter and copied to the ARS-Media Reports folder on the C drive. The report is in tab-delimited ASCII text (i.e., spreadsheet format).

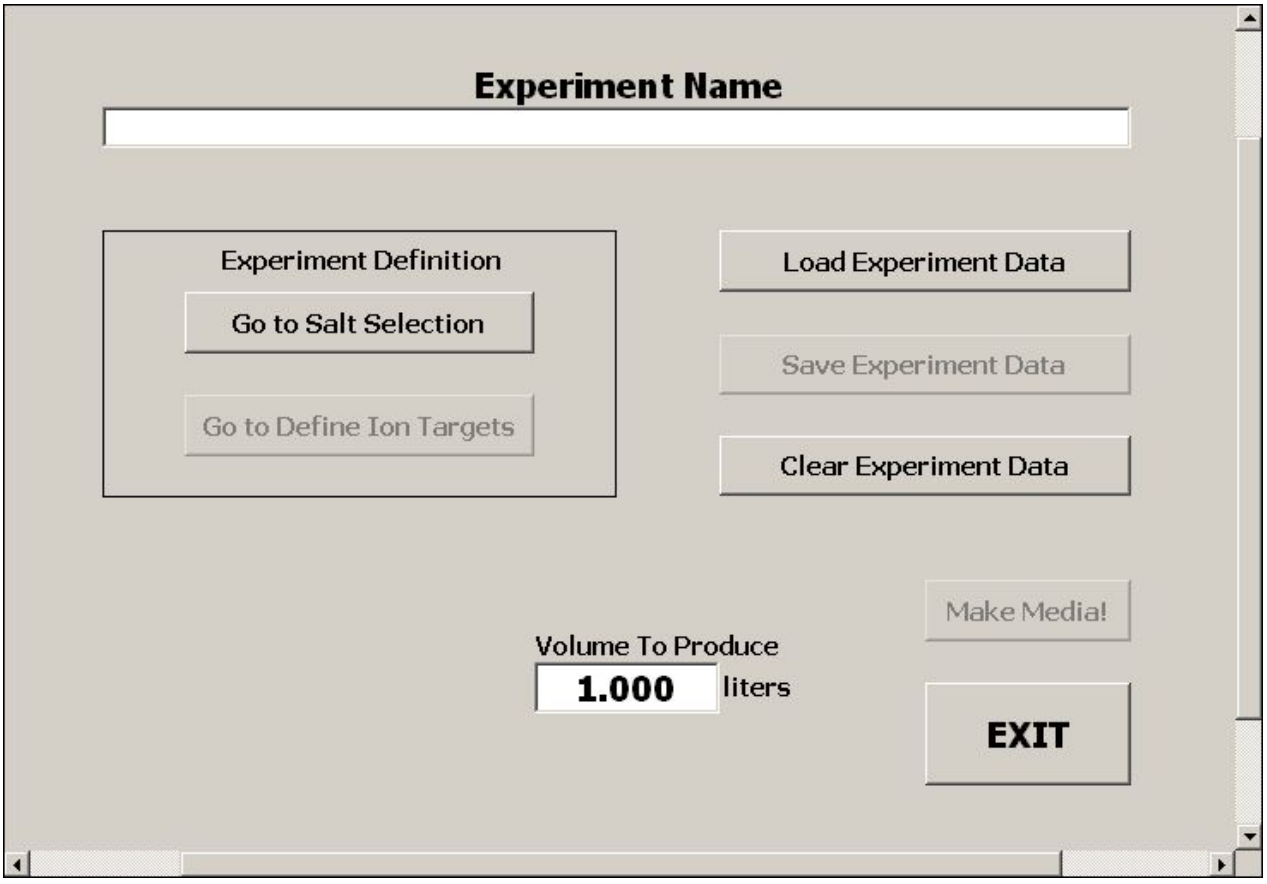
5. Salt selections and ion values can be saved using the Save Salt/Ion Settings button on the main panel. Previously saved values can then be loaded using the Load Salt/Ion Settings button on the main panel.

Precautionary Note

The formulations generated by ARS-Media are derived mathematically without regard to solution chemistry. The solution characteristics of an ARS-Media formulation (e.g., pH, dissolved species and precipitated solids) can be determined using a chemical equilibrium modeling program such as MINEQL+, MINTEQA2, and/or PHREEQC.

Main Panel

The program is started by going to Start/Programs/ARS-Media Calculator, or by using a desktop shortcut (if created by the user). The Main Panel of the ARS-Media program opens and appears as follows:



Most ARS-Media windows may be resized to fit the majority of monitors/resolutions by double clicking in the lower right-hand corner of each window.

The user has two methods for defining the information needed to calculate a salt recipe. One, existing salt/ion settings can be loaded from a previously saved file and the values modified or, two, the user can select “Go to Salt Selection” and enter all values manually.

Main panel controls & indicators:

Experiment Name – The user can type in text to name the experiment here. If the user loads previously defined salt/ion settings, this field is populated from the information in the file.

Go to Salt Selection – Clicking on this button opens the Salt Selection screen.

Go to Define Ion Targets – This button is disabled ("grayed out") until the user defines the salts to be used. Once salts have been selected (either via the Salt Selection screen, or by loading salt/ion settings), clicking on this button opens the Define Ion Targets screen.

Load Salt/Ion Settings – Clicking on this button opens a Windows navigation box where the user selects an experiment to load from a previously saved file. The starting directory path is C:\ARS-Media Experiments, but the user can choose to save & load salt/ion settings from other locations if desired. Once a file is selected, the values from the file populate the appropriate fields in the Salt Selection and Define Ion Target screens. The user can modify these values.

Save Salt/Ion Settings – Clicking on this button allows the user to navigate to a directory where the salt/ion settings can be saved. Again, the starting directory path is C:\ARS-Media Experiments, but the user can choose to save & load salt/ion settings in another location if desired. The file is saved in a form which can only be easily accessed in this program, not in a spreadsheet format.

Clear Salt/Ion Settings – Clicking on this button clears all settings and resets all values to their default levels.

Make Media! – This button is disabled until an experimental name is entered, salts have been selected, and ion targets defined. Once enabled, clicking on this button causes the program to execute the LP module to attempt a solution using the selected salts and defined ion target values. If a solution cannot be found, the user is notified. If a solution is found, the program will produce the Media Formulation Report, which is a tab-delimited ASCII (spreadsheet) file. This report file is located in the C:\ARS-Media Reports folder.

EXIT – Clicking on this button closes and exits the program.

Salt Selection Screen

If the user clicks on the "Go to Salt Selection" button on the main panel, the Salt Selection Screen opens.

The user selects/deselects the salts to use by pressing the green "Use?" buttons. Select All and Clear All buttons are provided for convenience. The Objective Function Coefficient (OFC) and Salt Concentration values for each salt are set at a default value of 1. The LP module will consider all selected salts equally when solving for a solution. There are two options if the user considers some salts less desirable (e.g., solubility, cost, availability, etc.) and would prefer that, if possible, the salt not be included in the solution. Option one - the user does not select the less desirable salt(s). The LP module only seeks a solution using the selected salts; salts not selected are ignored. Option two - the user enters a higher OFC value (e.g., 10) for the less desirable salts. The LP module will now attempt a solution using only the salts with the lowest OFC values, if a solution cannot be found, then the salts with the higher OFC value will be considered. OFC values can be any value ≥ 0.

If previously saved experimental salt/ion settings have been loaded, the user can modify the salts, Objective Function Coefficient values, and Salt Concentrations.

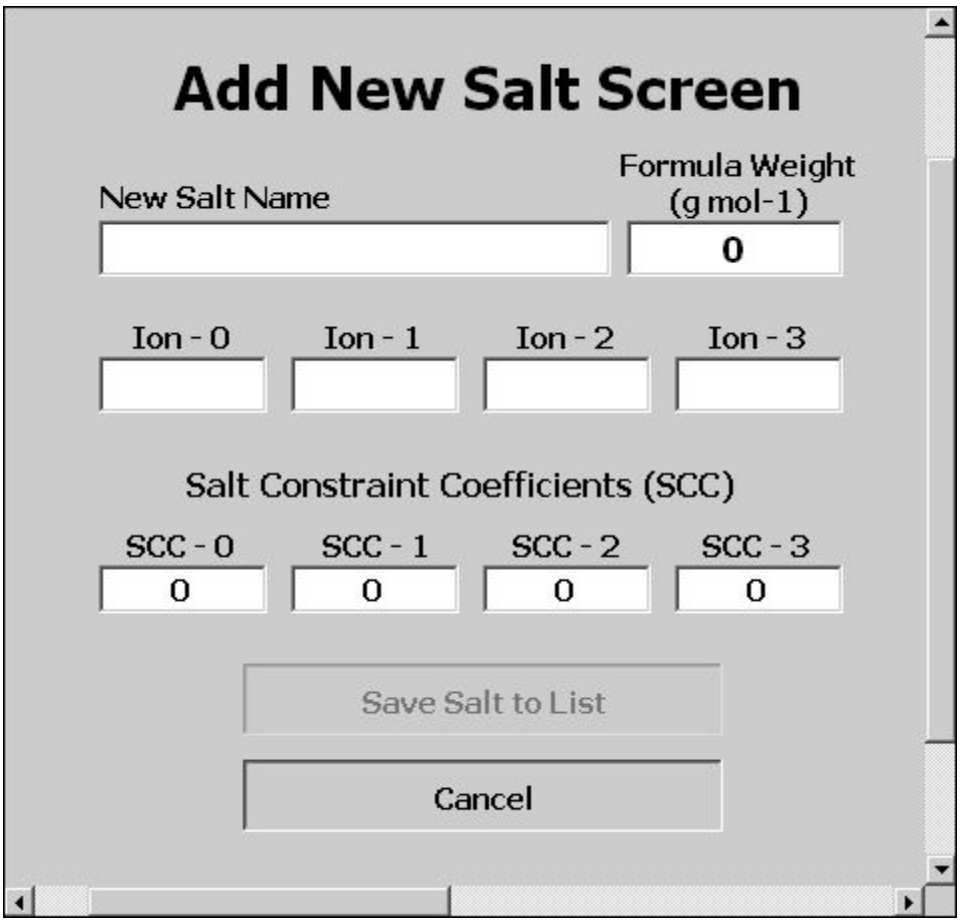
The Salt Names are populated from the Master Salt List file. The user can view the other salts by clicking on the up & down arrows next to the Salt Concentration column. The Master Salt List is a tab-delimited ASCII text file that lists salts, the mM value of 1 gram of the salt, and the mM value of each anion and cation from 1 gram of the salt. The user chooses the salts to use by clicking on the "Use?" button next to the desired salt, and can define the Objective Function Coefficient by changing the value in the column next to the desired salt.

When the user clicks on either the "Proceed to Define Ion Target Screen" or "Return to Main Panel", the chosen salts are used to populate the ions listed on the Define Ion Target screen.

Add New Salt Screen

A user can add additional salts not included in the Master Salt List by clicking on the "Add New Salt." However, the new salt is added to a new salt list. The Master Salt List cannot be modified by the user. The new salt list will include any selected salts plus the newly added salts. For example, selecting all of the salts in the Master Salt List and then adding an additional salt will result in a new list containing all of the salts in the Master Salt List plus the newly added salt. To add a new salt the salt is first named in the New Salt Name (e.g., Ca(NO3)2.4H2O or calcium nitrate tetrahydrate). The formula weight of the new salt is entered into the Formula Weight box (e.g., 236.14 for Ca(NO3)2.4H2O). The component ions are then entered (e.g., Ion - 0 = Ca; Ion - 1 = NO3). Ions that equilibrate with water are ignored (e.g., H and OH). The Salt Constraint Coefficients (SCC) are then added. A Sample Salt Constraint Coefficient Table was constructed for various salts, including Ca(NO3)2.4H2O, and is provided below for clarity. The SCC is derived by multiplying the ion's equivalence times the mM value of 1 gram of the salt. For example, the salt Ca(NO3)2.4H2O has one equivalent of Ca and two equivalents of NO3; therefore, the each ion's SCC is calculated by multiplying the ion's equivalent times the mM value of 1 gram of the salt. For Ca(NO3)2.4H2O the calculation is as follows: 1 gram of Ca(NO3)2.4H2O equals 4.2346 mM; therefore, the SCC for Ca equals 4.2346 (1 equivalent X 4.2346) and the SCC for NO3 equals 8.4692 (2 equivalents X 4.2346). The "Save Salt" button is disabled and grayed out until some text is put in the "New Salt Name" box, the "Formula Weight" box is changed to a value other than 0, text values for at least 2 ions are entered in the "Ion-0", & "Ion-1" boxes, and non-zero values are entered into the "SCC-0", & "SCC-1" controls.

The user will be prompted to save the new salt to either a previously saved salt list or to create a new list. Once a list is saved the list becomes active within the program. However, the user will be prompted when the program first opens to select the salt list to use. Salt lists cannot be selected once the program is running.

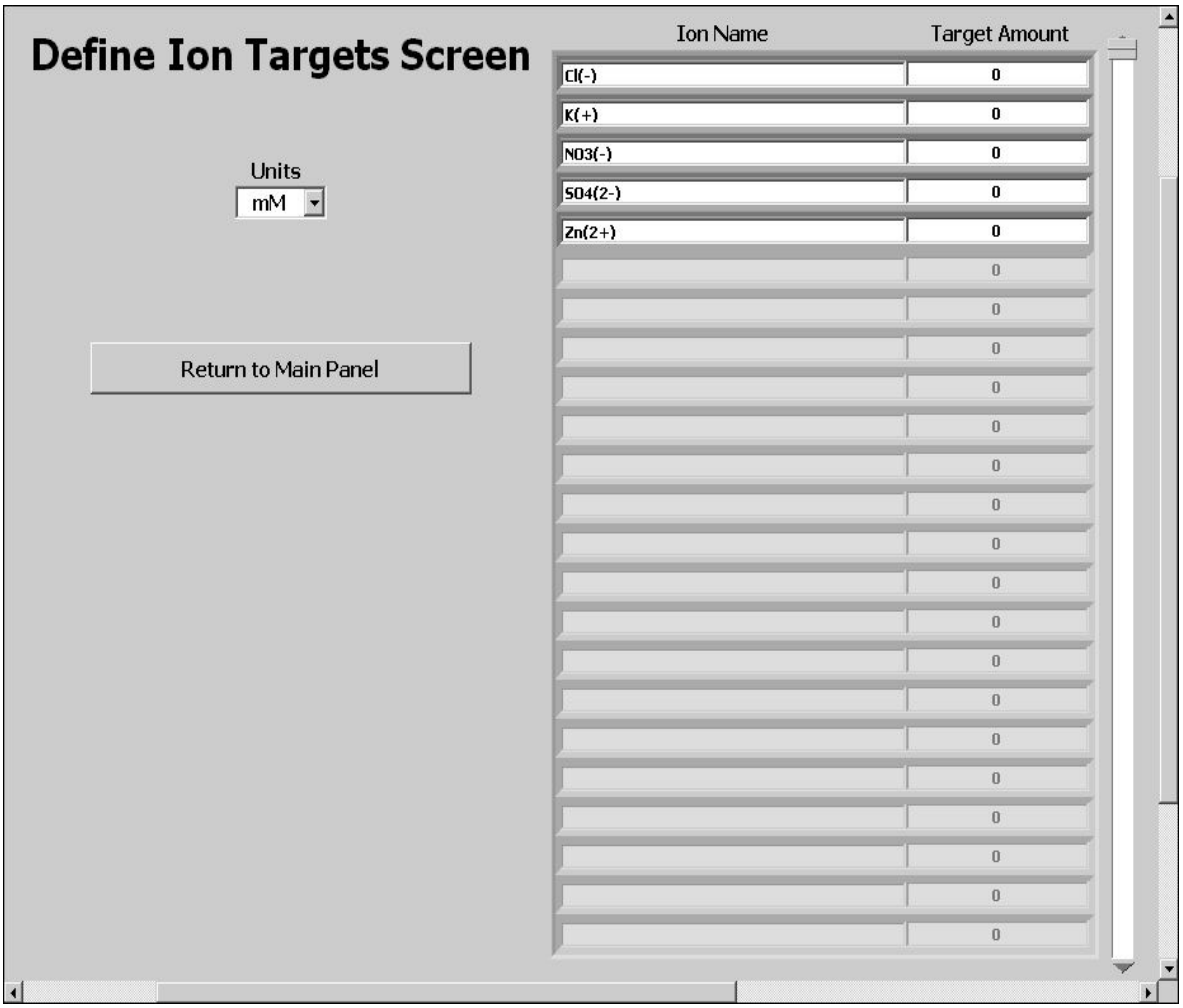


Sample Salt Constraint Coefficient Table[1]						
Salts	mM[2] (1 g)	Ions (mM)[3]				
		NO_3^-	Ca^{2+}	NH_4^+	PO_4^{3-}	K^+
KNO_3	9.8909	9.8909	0	0	0	9.8909
NH_4NO_3	12.4933	12.4933	0	12.4933	0	0
$(NH_4)_2 \cdot HPO_4$	7.5717	0	0	15.1434	7.5717	0
$Ca(NO_3)_2 \cdot 4H_2O$	4.2346	8.4692	4.2346	0	0	0
KCl	13.4136	0	0	0	0	13.4136
KH_2PO_4	7.3483	0	0	0	7.3483	7.3483

[1] A detailed explanation of the entire LP method is described in the Supplementary Methods section of Niedz, R.P. and Evens, TJ. A solution to the problem of ion confounding in experimental biology. Nature Methods 6:417, 2006.
[2] The mM value of 1 gram of the corresponding salt.
[3] The mM value of each anion and cation from 1 gram of the corresponding salt.

Define Ion Targets Screen

The Define Ion Targets Screen can be accessed from either the Main Panel or the Salt Selection screen. It appears as follows:



The Ion Names are populated from the salts selected on the Salt Selection screen. For example, three salts (KCl, KNO_3, and $ZnSO_4 \cdot 7H_2O$) were selected to generate the five ions pictured in the above screen. The user must define the target amount for each ion listed. The "Make Media!" button on the main panel will be disabled until at least one ion has a non-zero target amount.

The "Units" control determines the Target Amount units and allows a selection of µM, mM, or M. The default "Units" setting is mM. Please note that ARS-Media will automatically recalculate all Target Amounts as mM after leaving this screen. If this screen is revisited the Target Amounts will then be displayed in mM and ***not*** in the units originally specified.

Media Formulation Report

A report is generated if a solution is found after pressing the “Make Media!” button on the Main Panel. The report is a tab-delimited ASCII text file in spreadsheet format. This allows output to be readily used in other programs. The file is named using the name entered by the user in the “Experiment Name” along with a date and time (i.e. “Experiment Name YYYYMMDD HHMMSS.txt”). This avoids creating repetitive file names. The file contains complete information on the experiment, the salts selected, OFC values, stock concentrations for each salt, ion target values, and lastly the LP solution to make the requested ion solution. In the pictured report, the results should be understood as follows: The mls of a 1 mg/ml stock of each salt to add to a final volume of one liter. Since the stocks are all 1 mg/ml the solution can be understood also as mg/L of each salt. It should be noted that for KNO3 and NH4NO3 the mls required of 1424.865 and 1360.745, respectively, exceed one liter. Therefore, more concentrated stocks are required for these two salts. The concentration can either be adjusted in ARS-Media as previously explained and another report generated, or the concentrations can be easily adjusted in a spreadsheet. The report contains three sections. The first section includes summary information of the experiment, the salts selected, OFC values, and Concentration settings.

Experiment Name	MS
Date	28-Jul-06
Time	16:40:21

Experiment Parameters

Salt List File Name	Master Salt List.txt	
Volume (liters)	1	
Salts Chosen	OFC	Concentration (mg/ml)
CaCl2.2H2O	1	1
Ca(NO3)2.4H2O	1	1
KCl	1	1
KH2PO4	1	1
K2HPO4	1	1
KNO3	1	1
KOH	1	1
K4P2O7	1	1
K2SO4	1	1
KI	1	1
MgCl2.6H2O	1	1
Mg(NO3)2.6H2O	1	1
MgSO4.7H2O	1	1
NH4Cl	1	1
NH4H2PO4	1	1
(NH4)2HPO4	1	1
NH4NO3	1	1
(NH4)2SO4	1	1
CO(NH2)2	1	1

HNO3	1	1
H2SO4	1	1
H3PO4	1	1
NH4OH	1	1
MnSO4.4H2O	1	1
NaOH	1	1
NaCl	1	1
FeSO4	1	1
H3BO3	1	1
CuSO4.5H2O	1	1
CoCl2.6H2O	1	1
FeSO4.7H2O	1	1
Na2EDTA.2H2O	1	1
Na2MoO4.2H2O	1	1
NaFeEDTA	1	1
Ca(OH)2	1	1
Ca(SO4).2H2O	1	1
Ca(H2PO4)2.H2O	1	1
K3PO4	1	1
C4H6CaO4? H2O	1	1
CH3COOK	1	1
CH3COOH	1	1
HCl	1	1
Mg(OH)2	1	1
ZnSO4.7H2O	1	1
Zn(NO3)2.6H2O	1	1

The second section summarizes the ion target values.

Ions	Ion Target Value
B	0.1
Ca(2+)	3
Cl(-)	6
Co(2+)	0.0001
Cu(2+)	0.0001
EDTA	0.1
Fe(2+)	0.1
I(-)	0.005
K(+)	20
Mg(2+)	1.5
Mn(2+)	0.1
Mo(2-)	0.001
NH4(+)	20
NO3(-)	40
Na(+)	0.2
PO4(3-)	1.25
SO4(2-)	1.5
Zn(2+)	0.03

The third section includes the actual LP solution as mls/L for each salt stock. The objective function value of the LP solution is also provided. This can be useful for determining the least expensive formulation.

Results	
Objective Function Value	4.465
Salts	Amount (ml)
KCl	439.986
KNO3	1424.865
KI	0.83
NH4NO3	1376.754
(NH4)2SO4	184.974
HNO3	544.823
H3PO4	102.5
MnSO4.4H2O	22.305
NaCl	5.727
H3BO3	6.184
CuSO4.5H2O	0.025
CoCl2.6H2O	0.024
Na2MoO4.2H2O	0.242
NaFeEDTA	36.711
Ca(OH)2	222.27
Mg(OH)2	87.495
Zn(NO3)2.6H2O	8.923

Master Salt List File

The file that the program uses is found in C:\Program Files\USDA ARS Media Calculator\Master Salt List.txt. The Master Salt List cannot be modified. If salts are added or deleted the modified salt list must be saved as separate salt list file. The user is prompted upon opening the program to select the salt list file to use for the session. Selecting a different salt list file to use during a session is not possible; the user must close the program, restart ARS-Media, and then select the salt list of interest.

Software Support

Questions, comments, concerns or suggestions may be emailed to either of the following addresses:

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rniedz@ushrl.ars.usda.gov

Support will be somewhat limited, and may be sporadic. Hey, it's free software…

If you provide the authors with an email address/contact information we will endeavor to alert you to any updates or major happenings with ARS-Media.